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Research Article

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Characterization of the interaction between a Copper Complex and Bovine Serum Albumin: A Thermodynamic approach

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Abstract Interaction of Cu complex (Salen= N, N´-ethylene bis (salicylideneimine)) with Bovine Serum Albumin (BSA) was studied by absorption spectroscopy, competitive binding study and thermal denaturation study. The protein binding affinity of Cu complex was found to be ($3.0\times10^4 M^{-1}$). The binding plot obtained from the absorption titration data gives a binding constant of 3.5 ($\pm$ 0.1)$\times10^4$ M^{-1}. It was found that the charge transfer band of the metal complex was perturbed in the presence of BSA. The thermodynamic parameters ($\Delta H^o > 0$ and $\Delta S^o > 0$) showed that the hydrophobic interaction leads to the increasing entropy brought about by interaction with the complex. The negative ΔG^o values for interaction of BSA with the Cu complex indicate the spontaneity of the complexation. The thermodynamic parameters such as ΔG°_b, ΔH°_b, ΔS°_b were calculated by analyzing the UV/Vis data with a simple binding model. These thermodynamic parameters indicated that hydrophobic force play a major role in the binding.

Keywords Metal–Salen complex; Cu complex; Bovine Serum Albumin; protein-binding

Introduction

Study of binding of small molecules to proteins such **a**s HSA, BSA, LYS provides a data model, which provides information that leads to drug design based on a label for accurate quantification of proteins [1].

Small molecule binding to a protein such as a biosensor generates an important signal. In order to design appropriate metal complexes with potential applications in biology.

Pharmacology tendency to plasma proteins is an important factor that must be considered in the drug design .Since the effective concentration, nature and medical potential are strongly linked to their willingness them against specific binding sites on the carrier's bio- molecules , the issue of possible interactions between studied model of the drugs and carrier proteins is important. In the past decade , studies have shown that serum albumin in the blood plasma can be connected to a wide range of compounds such as phosphate, cysteine, glutathione ligands base shifts and the complexes of Cu (II), Ni (II), Mn (II), Co (II), Hg (II) and Zn (II) and thionin metal. HSA is one of the most widely studied proteins, which has provided a two-position link with high affinity for a variety of drugs IA, and IIIA can probably be replaced in the sequence [2].

A wide range of ions and molecules are connected to this protein with high affinity, and this protein carries them. Most drugs and metal complexes bind to serum albumin. This binding is a critical determinant of drug distribution, drug kinetics and the medicinal ability [2]. In order to design effective chemotherapeutic agents and better cancer drugs, studies on interaction of metal complexes with biomolecules are needed. Schiff base complexes, an important class of metal complexes, are in medical areas. In recent years this material as well as biological applications including anti-bacterial properties has shown excellent anti-fungal and anti- cancer properties. Di-amino ligands four teeth schiff base and its complexes are as biological models. Understanding biomolecular structure and processes

has been used in biology. Complexes interaction studies including a wide range of metal complexes study N_2O_2 ligands schiff base in many articles published in Biological Systems [2-3].

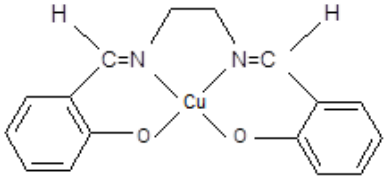
Schiff Base ligands are used for easy synthesis, low cost and a variety of side groups. Moreover, Schiff base ligands and metal complexes tend to interact with other proteins, addressing inhibition of enzyme activity. Inhibitors are used to complete with important pathophysiological conditions. On the other hand metal ions, particularly transition metal ions are a variety of coordinations and are primarily used to design appropriate coordination complexes. Nickel, as a transition metals ion, is an essential ingredient with very small amounts in bacteria, plants and animals and play an important role in the structure and activity of the urease enzyme [3].Serum albumin is the most abundant protein in the circulatory system of a wide variety of organisms and plays an important role in the transport and deposition of many drugs. Thus, an understanding of the features of drug interactions with albumin can provide insights into drug therapy and design. Consequently, attention has been focused on the binding of drugs to albumin. Bovine serum albumin (BSA) is usually employed as a model protein because of its low cost and availability and because it is structurally homologous with human serum albumin [4].

Salicylidene– ethylendiamine, often known as salen, is one of the most suitable ligands used for the production of metal catalysts. Metal–salen complexes are able to speed a wide range of reactions such as cyclopropanations, epoxidations and oxidations, and are valuable for kinetic resolutions [5]. These metal schiff base complexes have been developed as nucleic acid compounds to induce damages in DNA and/or RNA. Recently, synthetic salicylic aldehyde nucleosides have been used to improve the metal–salen-base pair complexes inside the DNA, and the complexation was found to repeal the succession information [6].

Binding of ligands to macromolecules is one of the most important reactions in biology. Often binding of one ligand molecule can enhance or reduce the receptor's affinity to bind subsequent ligand molecules. Such effects are in general referred to as "cooperative", and can be found in many biological systems. Reduction of affinity upon multiple ligand binding can be attributed to the presence of an effective repulsion between the ligands and is called negative cooperativity or anticooperativity. Affinity enhancement due to ligand binding can be attributed to an effective attraction between the ligands and is called positive cooperativity or simply cooperativity [6]. It is important to study the interaction of small ions and molecules, with macromolecules in order to understand the nature of transportation and distribution of these species in biological system because such interactions play a prominent role in transportation and distribution processes. In fact, most of biological functions have binding as a primary process. Binding of drugs, hormones, inhibitors etc. on to macromolecules has been widely studied [7].

To our best knowledge, the interaction of Cu complex with BSA has not been reported yet.

So,in the present work, we describe the synthesis and characterization of Cu complex (Scheme 1) and its interaction with BSA.This interaction has been investigated in view of thermodynamic using UV/Vis differential absorption.The aim of this study is to determine the binding constant, thermodynamic parameters and the mechanism and the kind of binding by analyzing absorption data using a simple binding model.



Scheme 1: Cu complex structure

Materials and Methods

Synthesis of Cu Complex

Salen ligand was synthesized by following the procedures established [7]. At first, 0.1 mol aliquot of ethylene diamine was dissolved in 25 mL of ethanol, and then this mixture was added to the solution of 0.2 mol of salicyl aldehyde in 150 mL of ethanol under stirring conditions. The obtained solution was refluxed for 1 h, and finally cooled down and kept at room temperature for 3 h. The formed yellow solid was filtered and recrystallized from ethanol. Cu complex was prepared using the following method: 0.01 mol of salen ligand was dissolved in 50 mL of

ethanol, and then the mixture was heated to boiling temperature. Then a solution of 0.01 mol of metal salt ($Cu(CH_3COOH)_2.4H_2O$) was added to 125 mL of ethanol. The resultant solution was stirred and refluxed for 1 h. After the solution was cooled to room temperature, the product was separated by filtration and recrystallized from CH_3OH.

Yield: 95%. Anal. Calc. for C16H14CuN2O2: C, 68.79; H, 6.87; N, 11.46. Found: C, 68.70; H, 6.81; N, 11.39. FT-IR (KBr) νmax cm^{-1}: 2931, 2795, 1626, 1456, 1288, 1154, 1014.

BSA was obtained from Sigma Chemical Co. HCl, K_2HPO_4, KH_2PO_4, NaOH and ethanol were from Merck Co. All of the other materials used were of analytical grade. Preparation of BSA (3.03×10^{-6} M) stock solution. Preparation of Cu complex (9.85×10^{-5} M) stock solution. Preparation of phosphate buffer 5mM, pH=7**.** Absorption titration experiments were carried out by varying the Cu(Salen) concentration and maintaining the BSA concentration constant in ethanol and 5 mM phosphate buffer, pH=7.

Apparatus:

Experiments were carried out by UV/Vis spectrophotometer model Perkin Elmer lambda 25

Result and Discussion:

The interaction of Cu complex with bovine serum albumin (BSA) in 5 mM phosphate buffer (pH=7) was studied by differential UV/Vis spectroscopy method at different temperature. The binding isotherms have been shown in Fig. 1 as the average number of bound ligands to one macromolecule of BSA (ν) versus $\ln[sa]_f$, where $[L]_f$ is the free ligand concentration.

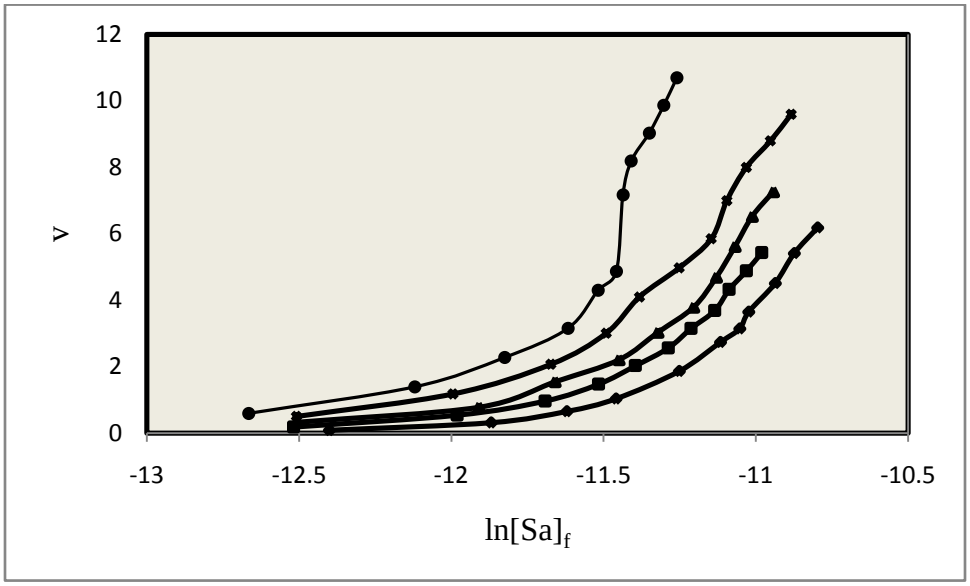


Figure 1: The binding isotherm of interaction BSA with Cu complex in 5 mM phosphate buffer solution, pH=7 at λ_{max} 352 andtemperatures at 25 (♦), 30(■), 35(▲), 40(∗), 45(●) ℃

The most common presentation of ligand-biomacromolecule binding data is the Scatchard plot. For a biomacromolecule which has *g* binding sites, and in which binding sites are characterized by identical intrinsic association binding constant, K_a, and independent of each other without interacting (that is, occupancy of one site does not affect the probability of binding to any other), from mass action equation, Scatchard showed that:

$$\frac{\nu}{[L]} = K_a(g - \nu) \quad (1)$$

For system with one set of binding sites and positive cooperativity in binding, the Scatchard plot should be downward as shown in Fig. 2[8].

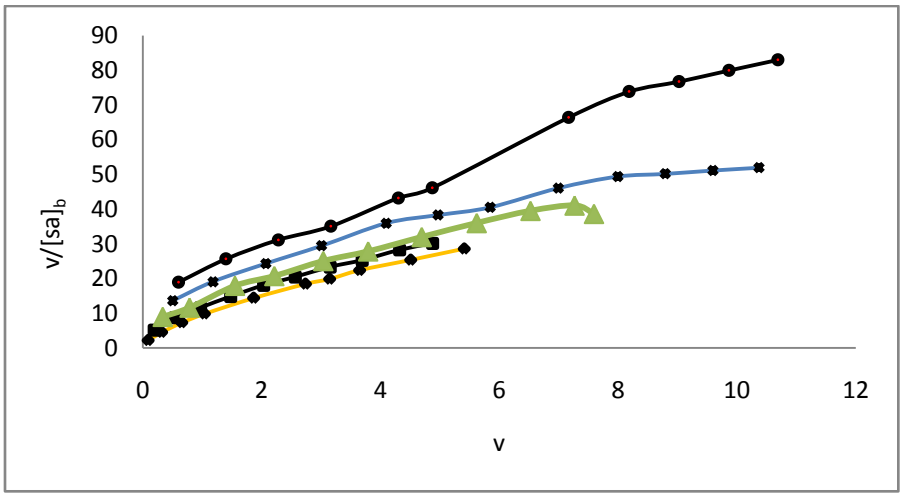


Figure 2: The scatchard plots of interaction BSA with Cu complex in 5 mM phosphate buffer solution, pH=7 at λmax 352 and temperatures at 25 (♦), 30(■), 35(▲), 40(∗), 45(●) ℃

For obtaining approximated values of binding parameters(Table 1), it might be possible to fit the binding data to Hill equation (2)[8].

$$\mathrm{Ln}\left(\frac{v}{g-v}\right) = \log K_b + n_H \,\mathrm{Ln}[L]_f \qquad (2)$$

Where g, K_b and n_H are the number of binding sites, binding constant and Hill coefficient, respectively.

The Hill plots obtained for the interaction of bovine serum albumin with Cu complex at temperature 25, 30, 35, 40 and 45°C are shown in Fig.3.

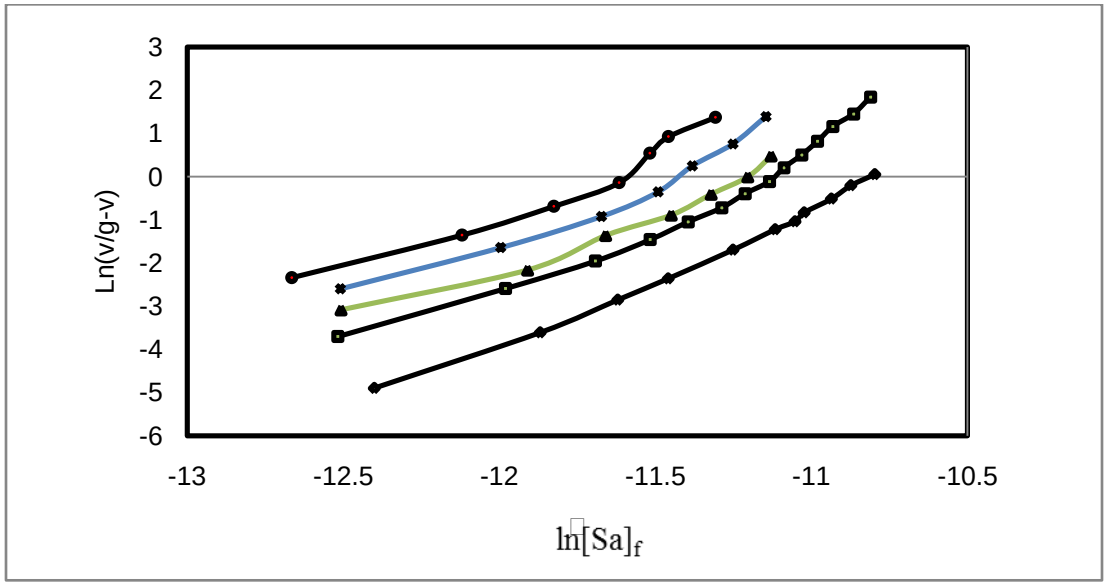


Figure 3: The Hill plot of interaction BSA with Cu complex in 5 mM phosphate buffer solution, pH=7 at λmax 352 and temperatures at 25 (•), 30((■, 35(▲(, 40(∗), 45(•) °C.

The van't Hoff (6) plot for interaction of Cu complex with BSA is shown in Fig.4.

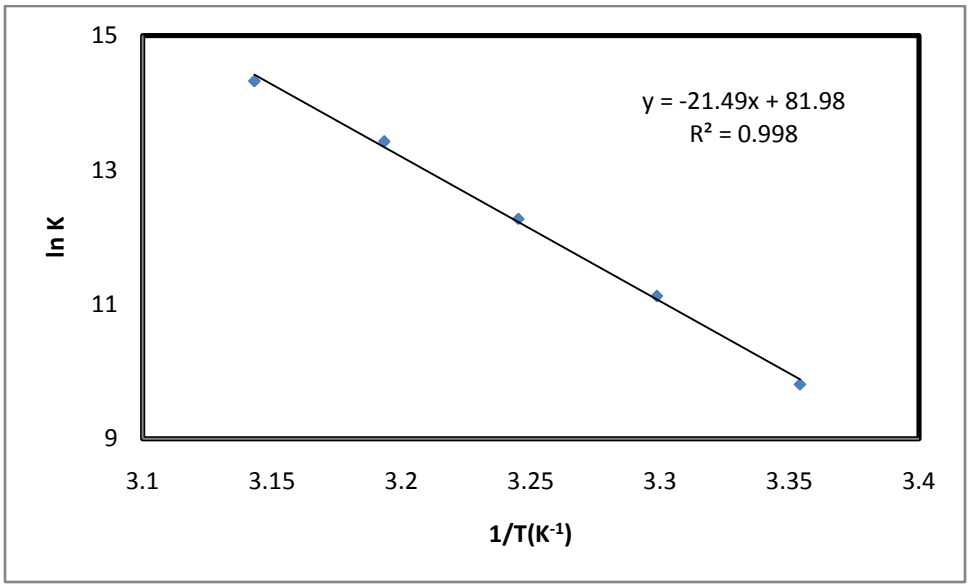


Figure 4: Van't Hoff diagram for BSA with Cu complex interaction in 5 mM phosphate buffer solution, pH=7 at different temperatures

Table 1: Binding constants K_b, n_H of interaction Cu(II) salen with BSA at 5 mM phosphate buffer, pH 7.0 and various temperatures.

t(°C)	n_H	lnK_b
25	2.96	9.81
30	2.90	11.12
35	2.89	12.27
40	2.84	13.42
45	2.64	14.32

From Table 1, n_H>1 indicating positive cooperativity. The calculated thermodynamic parameters for binding of Cu complex- BSA are listed in Table (2).

Then the Van't Hoff relation (3) was used for calculating the molar enthalpy of binding, ΔH_b°. Values of $K_b$were used to determine values of the molar Gibbs free energy, using equation (4). In addition, the molar entropies of ligand binding were also calculated, using equation (5) [9-10]. The calculated thermodynamic parameters for binding of Cu(II)Salen to BSA are listed in Table(2).

$$\frac{\partial \ln K_a}{\partial\left(\frac{1}{T}\right)} = -\frac{\Delta H_b^\circ}{R} \quad (3)$$

$$\Delta G_b^\circ = -RTlnKa \quad (4)$$

$$\Delta S_b^\circ = \frac{\Delta H_b^\circ}{T} - \frac{\Delta G_b^\circ}{T} \quad (5)$$

Table 2: Thermodynamic parameters for binding of Cu(II) Salen to BSA at 5 mM phosphate buffer, pH 7.0 and various temperatures

T(K)	ΔH_b° (KJ/mol)	ΔG_b° (KJ/mol)	ΔS_b° (J/mol)
298	178.58±6.61	-24.32±0.9	680.53±25.18
303		-28.03±1.04	681.54±25.22
308		31.43±1.16-	681.52±25.22
313		34.94±1.29-	681.85±25.23
318		-37.88±1.40	680.37±25.17

By analyzing the results of UV/VIS spectroscopy, the determined thermodynamic parameters ($\Delta H^{o} > 0$ and $\Delta S^{o} > 0$) showed that the interaction between BSA and Cu complex leads to the increasing enthalpy and entropy. The negative ΔG^{o} values for interaction of BSA with the Cu complex indicate the spontaneity of the complexation. Therefore the dominant force is entropy and the mode of this interaction is hydrophobic [10]. The binding and stage Gibbs free energy change of Cu complex–BSA is usually calculated by Eq.(6) in which [L] is free ligand concentration [8,9] .The Results are shown in Fig.5.

$$\Delta G^{\circ}_{b,v} = -RTn_H \ln K_a + RT(1-n_H)\ln[L] \quad (6)$$

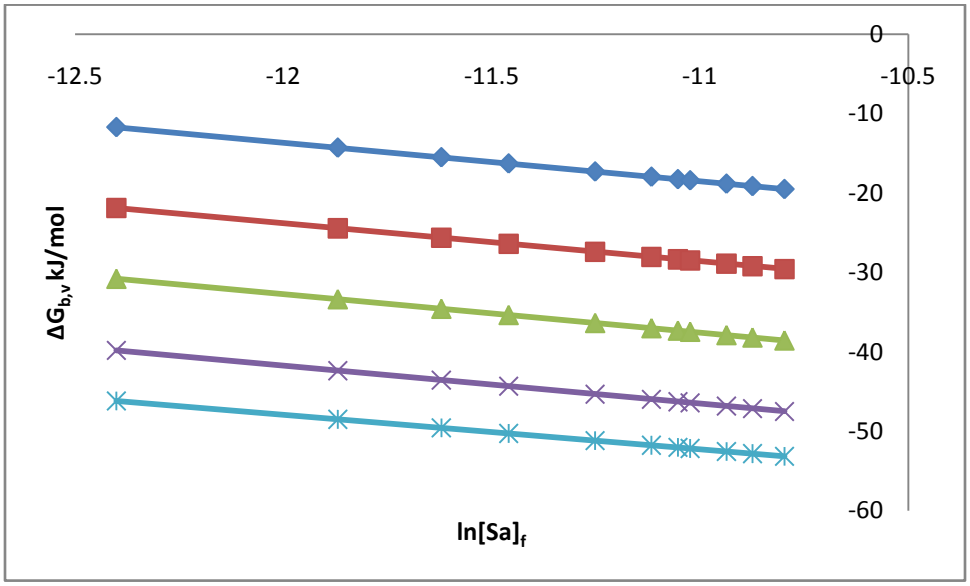


Figure 5: Binding and stage Gibbs free energy change of Cu complex–BSA complex formation in 5 mM phosphate buffer solution, pH=7 at λ_{max} 352 and temperatures at 25 (♦), 30(■), 35(▲), 40(×), 45(*)

Conclusion

In summary, we investigated the binding of BSA with a Cu complex. The thermodynamic parameters such as binding constant K_a, the binding gibbs free energy, ΔG°_b binding enthalpy changes, ΔH°_b binding entropy changes, ΔS°_b were calculated by analyzing the UV/Vis data with a simple binding model. With the obtained results of UV/vis spectroscopy, the thermodynamic parameters ($\Delta H^{o} > 0$ and $\Delta S^{o} > 0$) were determined. These results show that the interaction between BSA and Cu complex leads to an increasing enthalpy and entropy. The negative ΔG^{o} values for interaction of BSA with the Cu complex indicate the spontaneity of the complexation. Therefore, the dominant force is entropy and the mode of this interaction is hydrophobic[10].The binding isotherm , binding capacity and scatchard plots were plotted .The positive values of n_H show that BSA has one binding site set and presents positive cooperativity.

Acknowledgements

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